REMARKS

With entry of the instant amendment claims 15, 16, 18, 20 - 28, 30 - 51, 58 - 75 and 77 - 82 are pending in the application. Claims 29 and 76 have been canceled. Claims 80 - 82 have been added. Claims 15, 18, 20, 25, 26, 31, 47 – 51, 63, 73 and 77 - 79 have been amended.

The independent claims, 15, 18, 63 and 73 and new independent claim 80 include the limitation that not only is the oxidized and reduced form of the co-factor recycled between an oxidization step and reduction step wherein the reduction step forms 2-KLG, but also that the oxidation and reduction of the co-factor is coupled to said oxidation and reduction step. While the phrase "coupled to" is not explicitly used in the specification, it is clear from reading the specification that the regeneration of the oxidized and reduced form of the co-factor is not only recycled but also coupled to the oxidation of glucose and the reduction of 2, 5-DKG. Support for the phrase is found throughout the specification, and the Examiner's attention is directed to Figure 1, page 7, lines 9 - 14 and page 13, lines 22 - 30 of the disclosure. Additionally the independent claims now incorporate specific co-factors.

The recycling and coupling of the co-factor oxidation and reduction to the enzymatic oxidation and reduction step resulting in the production of 2-KGL is critical to the claimed invention. The present invention does not involve the introduction of a second substrate for co-factor regeneration. Prior art examples of enzymatic co-factor regeneration for *in vitro* conversion of biochemical substrates, which functioned as intermediates in ascorbic acid (ASA) production, required a separate balancing reaction in which a second product was generated.

Claim 15 has also been amended to recite that the carbon source is enzymatically oxidized by at least three enzymatic oxidative steps, and support is found in Figures 1 and 2 and in the original claims. The phrase "and enzymatic derivatives thereof" has been canceled from claims 15 and 18. The dependency of claims 20, 25, 26, 47 - 51 and 77 - 79 has been changed. Claim 31 has been amended to correct the preamble. New claim 80 is an independent claim and support is found in original claims 18, 19 and 21 and in Figure 1. Claims 81 and 82 further define the co-factor. Claims 77 - 79 now depend from new claim 80.

The Examiner has objected to claims 47 - 51 under 37 CFR 1.75(c) as being in improper form. The claims have been amended to correct the informality and now refer to other claims in the alternative.

Claims 15, 18, 20 and 25 - 32 have been rejected under 35 U.S.C. §112, second paragraph; claims 15, 16, 18, 21 - 24, 33 - 46 and 58 - 79 have been rejected under 35 U.S.C.

§112, first paragraph; claims 15, 16, 18, 21 - 24, 33 - 46 and 58 - 79 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Light et al., and claims 15, 16, 18, 21 - 24, 33 - 46 and 58 - 79 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Powers et al. Applicants respectfully request the withdrawal of each rejection based on the arguments and amended claims presented herein.

Rejections under 35 U.S.C. §112, second paragraph.

With respect to claims 15 and 18, the Examiner alleges it is unclear which "enzymatic derivatives thereof" are encompassed by the claims. This phrase has been deleted from said claims. Further claims 20 and 25 - 32 have been rejected as being incomplete because they depended from canceled claim 19. The claims have been amended to correct the oversight.

Rejections under 35 U.S.C. §112, first paragraph.

With respect to claims 15, 18 and 76 the Examiner states,

> "the claims encompass pathways from any carbon source to KLG through any intermediate........ The specification fails to describe other representative reactions that produce 2-KLG from any carbon source. Therefore, said reactions are characterized only by the final product, 2-KLG.

Applicants' claims are not directed to any carbon source but to a structurally related class of carbons that is 6-carbon sugars, a mixture of 6-carbon sugars and 6 -carbon sugar acids. While the reaction disclosed in the specification is the oxidation of glucose through various intermediates to 2-KLG, one skilled in the art is aware of various reactions, which could lead to the production of the intermediate 2-KLG. For example, 6-carbon sugars known to form 2-KLG include D-sorbitol, L-sorbose, 5-keto-D-gluconic acid, L-idonic acid and L-gulonic acid.

With respect to claims 63 and 73, the Examiner has indicated that while the claims specify the carbon source and the enzymatic activities, they do not specify cofactor. The claims have been amended to incorporate specific examples of cofactors. The Examiner further rejects the claims because the specific enzymes are described by function only, and concludes that the claims are drawn to a method of use of a genus of molecules described by broad function. Applicants submit a description of structural attributes for every enzyme that could be used in

the claimed process is not required. Members of the genus may be defined by their function. As stated by the Examiner and taught in Applicants' disclosure, the production of ASA intermediates is known and various fermentation strains of the Enterobacteriacea family are known and used in the art.

The Examiner further states with respect to claims 15, 16, 18, 21 - 24, 33 - 46 and 58 - 79,

> "the specification, while being enabling for producing 2-KLG from glucose using glucose dehydrogenase, gluconate dehydrogenase, 2-KDG dehydrogenase and reductase A:F22Y/A272G, does not reasonably provide enablement for producing 2-KLG from any carbon source using any oxidase and reductase."

Applicants again emphasize that claims 63, 73 and 80 specifically recite the carbon source as glucose and specifically recite glucose dehydrogenase as the oxidative enzyme. Additionally the claims are directed to the cofactors NAD/NADH or NADP/NADPH. With respect to claims 15 and 18 the phrase "enzymatic derivatives thereof" has been canceled and the term carbon source is defined by the Markush group. It is submitted that the specification does enable the scope of the claimed invention.

Rejections under 35 U.S.C. §103(a).

Claims 15, 16, 18, 21 - 24, 33 - 46 and 58 - 62 have been rejected as being unpatentable over Light et al. The Examiner states,

> Light et al. (US Patent 4,758,514) teach the pathway glucose-2-KLG (column 1, lines 16 - 29). They further teach the production of 2-KLG from glucose by Erwinia cell transformed with 2,5-DKG reductase gene (column 17, line 62 through column 20, line 5, Examples 5 and 6). This process comprises enzymatic oxidation of glucose by Erwinia into DKG and enzymatic reduction of DKG to 2-KLG. Since enzymes involved in oxidation of glucose to DKG are known in the art it would have been obvious to the one of ordinary skill in the art at the time the invention was made to carry out non-fermentative oxidation of glucose into DKG using purified enzymes or cells transformed with a DNA encoding an enzyme. One would have been motivated to use non-fermentative oxidation of glucose into DKG because it allows a more efficient and convenient production of larger quantities of DKG and KDG compared with the fermentative production."

Applicants assert Light et al. is concerned with an in vivo process. Moreover, Light et al. do not disclose a process for production of 2-KLG wherein at least one oxidative enzyme activity of the process requires an oxidized form of a co-factor and the reducing enzymatic activity of the process requires a reduced form of the co-factor wherein the reduced and oxidized co-factors are recycled between and coupled to said oxidizing step and reducing step. Recycling the oxidized and reduced form of the co-factor and the coupling of the reaction to the oxidization step and reduction step for regeneration is a critical element of the instant invention. This element is recited in each independent claim (claims 15, 18, 63, 73 and 80). At column 7, Light et al. discloses that the DKG reductase requires NADPH and that sources of electrons for the reduction of the coenzyme may be provided by any reduced substrate in contact with an enzyme for its oxidation, such as glucose/glucose dehydrogenase; glutamate/glutamate dehydrogenase; and formate/formate dehydrogenase. Further Light et al. disclose that other systems for regenerating NADPH cofactors are known in the art using, for example H_2 as a source of reducing equivalents and lipoamide dehydrogenase and hydrogenase or ferredoxin reductase and hydrogenase as catalysts. However, there is no teaching or suggestion that the process for the production of 2-KLG from a carbon source as disclosed in the instant specification could include a recycling and coupling of the co-factor between the oxidizing step and the reducing step such that co-factor is continually regenerated. One very significant advantage of the co-factor recycling of the claimed process is that enzymatic regeneration of the co-factor dependent reductase is not at the expense of another substrate that is oxidized. In the present invention, glucose can generate reducing power and still remain intact for conversion to 2KLG. This is not the case in the process as taught by Light et al. While glucose may be used for reducing power in Light et al., a portion of the glucose would breakdown to CO_2. Also as stated at page 11, lines 4 - 8 of the specification, this embodiment provides a means for co-factor regeneration, thereby eliminating the cost of continuously adding exogenous co-factor to the bioreactor for the production of KLG in Pantoea cells.

Additionally, claims 15, 16, 18, 21 - 24, 33 - 46 and 58 - 79 have been rejected as being unpatentable over Powers et al. The Examiner states,

> "Powers (US Patent 5,795,761) teach the pathway glucose-2-KLG. They further teach that a number of microorganisms such as *Erwinia*, *Acetobacter* and *Gluconobacter* can produce 2,5-DKG from glucose and the second group can reduce 2,5-DKG to 2-KLG (column 1. line 25 - 61). They teach reductase A:F22Y/A272G

> mutant (figure 10, for example) catalyzing conversion of 2,5-DKG to 2-KLG. Since enzymes involved in oxidation of glucose to DKG are known in the art it would have been obvious to one of ordinary skill in the art at the time the invention was made to carry out non-fermentative oxidation of glucose into DKG using purified enzymes or cells transformed with a DNA encoding an enzyme. One would have been motivated to use non-fermentative oxidation of glucose into DKG because it allows a more efficient and convenient production of larger quantities of DKG and KDG compared with the fermentative production."

Powers et al. is concerned with the production of DKG mutants with improved properties. One mutant characterized by Powers et al., is the double mutant F22Y/A272G which is also used by the Applicants in the present application. However, Powers et al. suffers from the same deficiency as Light et al. There is no teaching or suggestion in the reference concerning a process for the production of KLG wherein the oxidized form and the reduced form of the required co-factor for the reducing step and at least one oxidization step are recycled between and coupled to said steps.

Applicants assert the cited references offer no suggestion or motivation to provide a process for the production of KLG wherein the process includes the recycling of co-factor between at least one oxidizing step and the reducing step of said process and without the waste of an added co-factor. Moreover beyond looking to the cited references to determine if it suggests doing what the inventors in this case have done, one must also consider if the cited references provide the required expectation of success. Both the suggestion and expectation of success must be founded in the cited references and not in Applicants' disclosure. In this case both the suggestion and the expectation of success are lacking.

Applicants contend neither Powers et al. nor Light et al. taken alone or in combination render the claimed invention unpatentable and request withdrawal of all rejections under 35 U.S.C. §103(a).

In view of the foregoing, Applicants believe all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (650) 846-7620.

Respectfully submitted,

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Enc: Marked-up Version of the Amended Claims
Clean copy of claims

MARKED-UP VERSION OF THE AMENDED CLAIMS

15.(Twice amended) A process for the non-fermentative production of 2-KLG from a carbon source, comprising the following steps in any order,

a. enzymatically oxidizing the carbon source by at least **[one oxidative enzymatic activity]** **three enzymatic oxidative steps** to an oxidation product wherein **[said oxidative]** **the first** enzymatic **[activity]** **oxidation step** requires an oxidized form of an enzymatic co-factor; and

b. enzymatically reducing said oxidation product **[by at least one reducing enzymatic activity]** to 2-KLG wherein said **[reducing]** enzymatic **reduction** **[activity]** requires a reduced form of said enzymatic co-factor**,**

wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled between **[at least one]** **and coupled to the first** oxidizing step and **[at least one]** **the** reducing step **wherein the oxidized from of said co-factor is selected from the group consisting of $NADP^+$, NAD^+, ATP, ADP, FAD and FMN, wherein** **[and]** said carbon source is selected from the group consisting of 6-carbon sugars, mixtures of 6-carbon sugars, **and** 6-carbon sugar acids, **[and enzymatic derivatives thereof wherein said carbon source]** **and** is capable of being converted to an **ascorbic acid (**ASA**)** intermediate .

18.(Twice amended) A process for the non-fermentative production of 2-KLG from a carbon source, comprising the following steps in any order:

a. enzymatically oxidizing the carbon source by a first oxidative enzymatic activity to a first oxidation product;

b. enzymatically oxidizing the first oxidation product by a second oxidative enzymatic activity to a second oxidation product;

c. enzymatically oxidizing the second oxidation product by a third oxidative enzymatic activity to a third oxidation product; and

d. enzymatically reducing the third oxidation product by a **[reducing enzymatic activity]** **reductase enzyme** to 2-KLG wherein at least one of said first, second and third oxidative enzymatic activities requires an oxidized form of an enzymatic co-factor and said **[reducing enzymatic activity]** **reductase enzyme** requires a reduced form of said enzymatic co-factor**,**

[and] wherein the oxidized form and the reduced form of said co-factor are recycled between **and coupled to** at least one oxidizing step and the reducing step **wherein the oxidized from of said co-factor is selected from the group consisting of $NADP^+$, NAD^+, ATP, ADP, FAD and FMN,** and **wherein** said carbon source is selected from the group consisting of 6-carbon sugars, mixtures of 6-carbon sugars, 6-carbon sugar acids, **[and enzymatic derivatives thereof wherein said carbon source]** **and** is capable of being converted to an **ascorbic acid (**ASA**)** intermediate .

20.(Once amended) The process of **[Claim 19]** **Claim 18** wherein said first oxidative enzymatic activity requires an oxidized form of said enzymatic co-factor.

25.(Once amended) The process of **[Claim 19]** **Claim 18** wherein at least one of said first, said second, said third and said fourth enzymatic activities are immobilized.

26.(Once amended) The process of **[Claim 19]** **Claim 18** wherein at least one of said first, said second, said third and said fourth enzymatic activities are in solution.

31.(Once amended) **[The reductase activity]** **The process** of Claim 29 wherein said **[source includes]** **reductase activity is obtainable from** Corynebacterium or Erwinia.

47.(Twice amended) The process of **[Claims 15 and 18]** **Claim 15 or Claim 18** that is continuous.

48.(Twice amended) The process of **[Claims 15 and 18]** **Claim 15 or Claim 18** that is batch.

49.(Twice amended) The process of **[Claims 15 and 18]** **Claim 15 or Claim 18** that proceeds in an environment comprising organic solvents.

50.(Twice amended) The process of **[Claims 15 and 18]** **Claim 15 or Claim 18** that proceeds in an environment comprising long polymers.

51.(Twice amended) The process of **[Claims 15 and 18]** **Claim 15 or Claim 18** further comprising the step of obtaining ASA from said 2-KLG.

63.(Once amended) A process for the non-fermentative production of 2-KLG from **[a carbon source]** **glucose** comprising the following steps:

a. enzymatically oxidizing glucose by a glucose dehydrogenase to gluconate;

b. enzymatically oxidizing gluconate by a gluconic acid dehydrogenase to 2-KDG;

c. enzymatically oxidizing 2-KDG by a 2-KDG dehydrogenase to 2,5-DKG; and

d. enzymatically reducing 2,5-DKG by a 2,5-DKG reductase to 2-KLG

wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor and said reductase requires a reduced form of said enzymatic co-factor and the oxidized co-factor and the reduced-cofactor are recycled between **and coupled to** the glucose oxidizing step and the reducing step **and wherein the oxidized form of said co-factor is $NADP^+$ or NAD^+**.

73.(Once amended) A process for the non-fermentative production of 2-KLG from **[a carbon source]** **glucose** comprising the following steps:

a. enzymatically oxidizing glucose by a glucose dehydrogenase to gluconate;

b. enzymatically oxidizing gluconate by a gluconic acid dehydrogenase to 2-KDG;

c. enzymatically oxidizing 2-KDG by a 2-KDG dehydrogenase to 2,5-DKG; and

d. enzymatically reducing 2,5-DKG by a 2,5-DKG reductase to 2-KLG

wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor and said reductase requires a reduced form of said enzymatic co-factor and the oxidized co-factor and the reduced-cofactor are recycled between **and coupled to** the glucose oxidizing step and the reducing step**, and wherein the oxidized form of said co-factor is $NADP^+$ or NAD^+**

wherein the process proceeds in an environment wherein the 2,5-DKG reductase is provided exogenously to a host cell.

77.(Once amended) The process of **[Claim 76]** **Claim 80** wherein the host cells are viable.

78.(Once amended) The process of **[Claim 76]** **<u>Claim 80</u>** wherein the host cell is non-viable.

79.(Once amended) The process of **[Claim 76]** **<u>Claim 80</u>** wherein the host cell is modified to eliminate the naturally occurring GDH activity and a heterologous GDH having a specificity for $NADP^+$ or NAD^+ is introduced into said host cell.

CLEAN CLAIM SET

Claims 1 - 14 - canceled

15.(Twice amended) A process for the non-fermentative production of 2-KLG from a carbon source, comprising the following steps in any order,

a. enzymatically oxidizing the carbon source by at least three enzymatic oxidative steps to an oxidation product wherein the first enzymatic oxidation step requires an oxidized form of an enzymatic co-factor; and

b. enzymatically reducing said oxidation product to 2-KLG wherein said enzymatic reduction requires a reduced form of said enzymatic co-factor,

wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled between and coupled to the first oxidizing step and the reducing step wherein the oxidized from of said co-factor is selected from the group consisting of $NADP^+$, NAD^+, ATP, ADP, FAD and FMN,

wherein said carbon source is selected from the group consisting of 6-carbon sugars, mixtures of 6-carbon sugars, and 6-carbon sugar acids, and is capable of being converted to an ascorbic acid (ASA) intermediate.

16.(Reiterated) The process of Claim 15 wherein said carbon source is KDG.

17. canceled

18.(Twice amended) A process for the non-fermentative production of 2-KLG from a carbon source, comprising the following steps in any order:

a. enzymatically oxidizing the carbon source by a first oxidative enzymatic activity to a first oxidation product;

b. enzymatically oxidizing the first oxidation product by a second oxidative enzymatic activity to a second oxidation product;

c. enzymatically oxidizing the second oxidation product by a third oxidative enzymatic activity to a third oxidation product; and

d. enzymatically reducing the third oxidation product by a reductase enzyme to 2-KLG

wherein at least one of said first, second and third oxidative enzymatic activities requires an oxidized form of an enzymatic co-factor and said reductase enzyme requires a reduced form of said enzymatic co-factor, wherein the oxidized form and the reduced form of said co-factor are recycled between and coupled to at least one oxidizing step and the reducing step
wherein the oxidized from of said co-factor is selected from the group consisting of $NADP^+$, NAD^+, ATP, ADP, FAD and FMN, and
wherein said carbon source is selected from the group consisting of 6-carbon sugars, mixtures of 6-carbon sugars, 6-carbon sugar acids, and is capable of being converted to an ascorbic acid (ASA) intermediate.

19. Canceled

20.(Once amended) The process of Claim 18 wherein said first oxidative enzymatic activity requires an oxidized form of said enzymatic co-factor.

21.(Reiterated) The process of Claim 18 wherein said carbon source is glucose and said first enzymatic activity is a glucose dehydrogenase activity.

22.(Reiterated) The process of Claim 21 wherein said glucose dehydrogenase activity is obtainable for a bacterial;, yeast or fungal source.

23.(Reiterated) The process of Claim 22 wherein said glucose dehydrogenase activity is obtainable from a source including T. acidophilum, Cryptococcus uniguttalatus and Bacillus species.

24. (Once amended) The process of Claim 18, wherein each of said first enzyme, said second enzyme and said third enzyme has dehydrogenase activity.

25.(Once amended) The process of Claim 18 wherein at least one of said first, said second, said third and said fourth enzymatic activities are immobilized.

26.(Once amended) The process of Claim 18 wherein at least one of said first, said second, said third and said fourth enzymatic activities are in solution.

27.(Once amended) The process of Claim 25 wherein said second enzyme has GADH activity.

28.(Once amended) The process of Claim 25 wherein said third enzyme has KDGDH activity.

29.(Once amended) The process of Claim 25 wherein said fourth enzyme is a reductase enzyme.

30.(Reiterated) The process of Claim 29 wherein said reductase activity is obtainable from a bacterial, yeast or fungal source.

31.(Once amended) The process of Claim 29 wherein said reductase activity is obtainable from Corynebacterium or Erwinia.

32.(Reiterated) The process of Claim 31 wherein said reductase activity is 2,5 DKG reductase.

33.(Reiterated) The process of Claim 18 wherein said first oxidation product is gluconate, said second oxidation product is 2-KDG, and said third oxidation product is 2,5-DKG.

34.(Reiterated) The process of Claim 18 that proceeds in an environment comprising recombinant host cells.

35.(Reiterated) The process of Claim 34 wherein said host cell is viable.

36.(Once amended) The process of Claim 34 wherein said host cell is non-viable.

37.(Reiterated) The process of Claim 34 wherein said recombinant host cells comprises members of Enterobacteriacea.

38.(Reiterated) The process of Claim 34 that proceeds in an environment comprising recombinant host cell membranes and wherein at least one of said first, said second and said third enzymes are bound to said host cell membranes.

39.(Reiterated) The process of Claim 37 wherein said recombinant host cell is a Pantoea species.

40.(Reiterated) The process of Claim 39 wherein said recombinant host cell is Pantoea citrea.

41.(Reiterated) The process of Claim 40 wherein said recombinant host cell has a mutation of at least one naturally occurring dehydrogenase activity.

42.(Reiterated) The process of Claim 41 wherein said mutation is in a membrane bound GDH activity.

43.(Reiterated) The process of Claim 41 wherein said host cell further comprises nucleic acid encoding a heterologous GDH activity.

44.(Reiterated) The process of Claim 43 wherein said heterologous GDH activity is obtainable from T. acidophilum, Cryptococcus uniguttalatus, or a Bacillus species.

45.(Reiterated) The process of Claim 18 wherein said oxidized form of said enzymatic cofactor is $NADP^+$ and said reduced form of said enzymatic cofactor is NADPH.

46.(Reiterated) The process of Claim 18 wherein said oxidized form of said enzymatic cofactor is NAD^+ and said reduced form of said enzymatic cofactor is NADH.

47.(Twice amended) The process of Claim 15 or Claim 18 that is continuous.

48.(Twice amended) The process of Claim 15 or Claim 18 that is batch.

49.(Twice amended) The process of Claim 15 or Claim 18 that proceeds in an environment comprising organic solvents.

50(Twice amended) The process of Claim 5 or 18 that proceeds in an environment comprising long polymers.

51.(Twice amended) The process of Claim 5 or 18 further comprising the step of obtaining ASA from said 2-KLG.

Claims 52 - 57 - canceled

58.(Once amended) The process of Claim 15 or Claim 18 wherein said 2-KLG is further purified via electrodiaysis.

59.(Reiterated) The process of Claim 45 or 46 wherein said cofactor is purified via nanofiltration.

60. (Reiterated) The process of Claim 18 that proceeds in an environment comprising salt.

61.(Reiterated) The process of Claim 60 wherein the salt includes ammonium sulfate, sodium acetate, ammonium acetate, ammonium chloride, sodium sulfate, potassium phosphate, sodium phosphate, sodium chloride, KCl, NH_4Cl, K_2SO_4, and NaI.

62.(Reiterated) The process of Claim 60 that comprises a salt concentration between 0 mM and 500 mM.

63.(Once amended) A process for the non-fermentative production of 2-KLG from glucose comprising the following steps:

a. enzymatically oxidizing glucose by a glucose dehydrogenase to gluconate;
b. enzymatically oxidizing gluconate by a gluconic acid dehydrogenase to 2-KDG;
c. enzymatically oxidizing 2-KDG by a 2-KDG dehydrogenase to 2,5-DKG; and
d. enzymatically reducing 2,5-DKG by a 2,5-DKG reductase to 2-KLG

wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor and said reductase requires a reduced form of said enzymatic co-factor and the oxidized co-factor and the reduced-cofactor are recycled between and coupled to the glucose oxidizing step and the reducing step, and wherein the oxidized form of said co-factor is $NADP^+$ or NAD^+.

64.(Reiterated) The process of Claim 63 wherein the oxidized form of said co-factor is NAD^+ or $NADP^+$ and said reduced form of said co-factor is NADH or NADPH.

65.(Reiterated) The process of Claim 63 wherein said 2,5-DKG reductase is obtainable from a bacterial, yeast or fungal source.

66.(Reiterated) The process of Claim 63 that proceeds in an environment comprising exogenously added 2,5-DKG reductase.

67.(Reiterated) The process of Claim 63 wherein any one of the dehydrogenases are obtainable from a bacterial, yeast or fungal source.

68.(Reiterated) The process of Claim 63 that proceeds in an environment comprising recombinant host cells.

69.(Reiterated) The process of Claim 68 wherein said recombinant host cells comprise members of Enterbacteriaces.

70.(Reiterated) The process of Claim 69 wherein said recombinant host cell is a Pantoea species.

71.(Reiterated) The process of Claim 68 wherein the host cell comprises a nucleic acid encoding a heterologous 2,5-DKG reductase.

72.(Reiterated) The process of Claim 68 wherein the host cell comprises a nucleic acid encoding a heterologous glucose dehydrogenase.

73.(Once amended) A process for the non-fermentative production of 2-KLG from glucose comprising the following steps:

a. enzymatically oxidizing glucose by a glucose dehydrogenase to gluconate;

b. enzymatically oxidizing gluconate by a gluconic acid dehydrogenase to 2-KDG;

c. enzymatically oxidizing 2-KDG by a 2-KDG dehydrogenase to 2,5-DKG; and

d. enzymatically reducing 2,5-DKG by a 2,5-DKG reductase to 2-KLG

wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor and said reductase requires a reduced form of said enzymatic co-factor and the oxidized co-factor and the reduced-cofactor are recycled between and coupled to the glucose oxidizing step and the reducing step, and

wherein the oxidized form of said co-factor is $NADP^+$ or NAD^+,

wherein the process proceeds in an environment wherein the 2,5-DKG reductase is provided exogenously to a host cell.

74.(Reiterated) The process of Claim 73 wherein the oxidized form of said co-factor is NAD^+ or $NADP^+$ and said reduced form of said co-factor is NADH or NADPH.

75.(Reiterated) The process of Claim 74 wherein the host cell is obtained from a Pantoea species and the host cell is modified to eliminate the naturally occurring GDH activity.

76. Canceled

77.(Once amended) The process of Claim 80 wherein the host cells are viable.

78.(Once amended) The process of Claim 80 wherein the host cell is non-viable.

79.(Once amended) The process of Claim 80 wherein the host cell is modified to eliminate the naturally occurring GDH activity and a heterologous GDH having a specificity for $NADP^+$ or NAD^+ is introduced into said host cell.

80.(New) A process for the non-fermentative production of 2-KLG in an environment comprising host cells, comprising the following steps in any order,

a. enzymatically oxidizing glucose by a glucose dehydrogenase to produce a first oxidation product, wherein said oxidation requires an oxidized form of an enzymatic co-factor;

b. enzymatically oxidizing said first oxidation product to produce a second oxidation product;

c. enzymatically oxidizing said second oxidation product to produce a third oxidation product; and

d. enzymatically reducing said third oxidation product to 2-KLG , wherein said reduction requires a reduced form of said enzymatic co-factor

wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled between and coupled to the first oxidizing step and the reducing step and said oxidized co-factor is NAD^+ or $NADP^+$ and said reduced co-factor is NADH or NADPH.

81.(New) The process of Claim 80 wherein the oxidized co-factor is NAD^+ and the reduced co-factor is NADH.

82.(New) The process of Claim 80 wherein the oxidized co-factor is $NADP^+$ and the reduced co-factor is NADPH.